

Solation of Citric Acid from Microorganisms

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Annotation: Citric acid is widely used in medicine, food, chemical and light industries. According to the data, the world production of citric acid is 400 thousand tons per year. This article describes the fermentation steps in citric acid production, industrial synthesis, shallow culture, and liquid culture.

Keywords: citric acid, aspergillus niger strain, molasses, fermentation, neutralization, culture liquid, calcium citrate, calcium oxalate, calcium gluconate.

INTRODUCTION. Citric acid is widely used in medicine, food production, chemical and light industries. According to the data, the global production of citric acid is 400 thousand tons per year. The large-scale production of citric acid was achieved only after it was confirmed that hydrocarbons and hydrocarbons could be used as a source of carbon in the process. [1]

Producer microorganisms of citric acid are microscopic fungi (*Aspergillus niger*), yeasts (*Candida lipolytica*, *Candida quilliermondii*) and bacteria (*Corynebacterium*, *Arthrobacter*).

LITERATURE ANALYSIS AND METHODOLOGY. In Russia, citric acid is obtained on the basis of microbiological synthesis by growing the microscopic fungus *Aspergillus niger* in a molasses medium. The process of citric acid production includes all the main stages of microbiological technology:

Obtaining planting material;

Molasses - preparation of raw materials for fermentation;

Air preparation and sterilization;

Fermentation;

Separation of mycelial fungus-producer biomass;

DISCUSSION. Separation of citric acid from the culture fluid and crystallization.

Producers that synthesize citric acid can be propagated by inoculation on the surface, surface, and liquid. The technological scheme of production of citric acid in these methods differs only in the fermentation stage. All other steps are the same. [2]

Experimental part: Preparation of planting material:

Aspergillus niger strains stored in special microbiological museums are stored in the form of dry spores (conidia) in a mixture of activated charcoal. The first culture is developed in agar medium in test tubes, and then grown in solid medium in tubes and cuvettes. The growing temperature is 32 ° C and the duration of cultivation is 2 to 7 days at each stage.

When grown on the surface of a solid nutrient medium, a conidial mycelial coating develops.

The mature conidia are collected using a vacuum equipment. The collected conidia are mixed with sterile additives (talc or activated charcoal) and dried at 32 ° C. The finished planting material is packed in sterile glass tubes or jars with a capacity of 0.5 to 1 liter. The shelf life of planting material treated in this way is not less than 6 months.

Preparation of raw materials. Molasses, a residual product of sugar production, was adopted as a substrate for the production of citric acid on an industrial basis. Molasses is a raw material that does not have a clear standard (content). Therefore, its suitability for production is determined after a small amount of controlled fermentation in the laboratory.

Quality molasses contains less than 46% unknown sugar. If the release of citric acid during the control fermentation process is 1.25 kg / (m² • milk) by surface injection or 12 kg / (m³ • milk) by liquid injection, such molasses is considered suitable for production.

Fermentation by the method of cultivation on the surface of the nutrient medium. The nutrient medium is prepared by boiling in special pots to grow on the surface. Molasses is diluted 1: 1 with water and sulfuric acid is added to bring the pH of the solution to 6.8-7.2. Potassium hexacyanoferrate (GSFK) is added to a certain amount of yellow blood salt solution during boiling to precipitate iron salts and heavy metals.

Sources of nitrogen, phosphorus (potassium phosphate), macro and microelements (zinc, magnesium, potassium, etc.) are added to the molasses solution at a temperature of 60-70 ° C, one after the other. The prepared medium is transferred to a sterile container at a temperature of 45-50 ° C. The sugar content of the feed should be 12-16%.

The main fermentation is carried out on special racks (shelves) with closed compartments where the cuvettes are located. The cuvettes are made of right-angled aluminum or stainless steel. The cuvettes can be up to 7 m long, 1.8 m wide and up to 20 cm high. The cuvettes are filled with nutrient medium and the culture fluid is leaking through the nozzle to the bottom of the cuvette. The chamber is equipped with a heated sterile air delivery system. Prior to the new fermentation cycle, the chambers and cuvettes are cleaned and sterilized with a mixture of paraformalin and then degassed in a paroammiac mixture.

The medium is poured into sterilized and cooled chamber cuvettes in layers of 12 to 18 cm. *Aspergillus niger* conidia, i.e. planting material, are sprayed into the nutrient medium on special equipment. One day after planting, a thin white-yellow mycelial coating is formed, and three days later it thickens and shows a wrinkled, layered structure. Active growth of fungal mycelium is ensured at very low aeration, at a temperature of 34-36 ° C. [3]

During the phase of active acid formation, the temperature decreases to 32-34 ° C, and the air transfer is increased 3-4 times. The air supplied to the chamber is gradually reduced to prevent a decrease in the rate of acid formation and a decrease in the amount of heat released.

The fermentation process is stopped when 1-2% of sugar is left in the solution and the amount of acid in the culture liquid is 12-20%. The culture liquid from the cuvettes is poured into the product collector and then transferred to the chemical plant. There citric acid is separated. The retention of citric acid in the culture fluid is 12-20%. It is washed from mycelial acids by washing with hot water and only then can it be used as feed for cattle.

Fermentation by growing in a liquid medium. The process of obtaining citric acid by growing *Aspergillus niger* fungi in liquid feed is carried out in fermenters with a volume of 100 m³. Growing mycelium obtained from fermenters with a volume of 10 m³ is used as planting material.

The molasses solution is controlled for sowing and production fermenters in the same way as the surface-grown method, except that the amount of sugar in the initial molasses solution for fermentation in the liquid should not be less than 4%. If the sugar content decreases sharply during the fermentation process, this indicator is brought to the norm using a sterile molasses solution containing 25-28% sugar. This solution is added until the total amount of sugar in the

fermenter reaches 12-15%.

Conidia suspension, initially stored in a thermostat at a temperature of 32 ° C for 5-6 hours, is first poured into a culture medium filled with nutrient medium. The culture is grown at a temperature of 34-35 ° C with constant stirring and aeration. During the cultivation process, the air supply to the fermenter is strictly controlled, i.e. the air consumption increases almost 10 times towards the end of fermentation.

During intensive foaming, a small amount (foaming agent) of foaming inhibitors (oleic acid) is added.

The maturation process of the mycelium takes 30-36 hours. During this period, the amount of citric acid in the culture fluid is 1-2% and the process is stopped. The mature mycelium is sent to the nutrient medium in the production fermenter.

The process of acid formation in the fermenter is continuous aeration and lasts 5-7 days at a temperature of 31-32 ° C. Air consumption increases to 400m³ / s in the initial period and to 2200m³ / s at the end of fermentation. To keep the amount of sugar moderate, the infusion solution is added 2-3 times from time to time. In this case, the total amount of sugar in the solution should be 12-15%. At the end of the process, the total acidity and sugar content are determined.

After the fermentation process is completed, the culture liquid is heated in a sharp steam at a temperature of 60-65 ° C and poured into the collector. From there, the mycelial biomass is transferred to a vacuum filter for washing and separation. Washed mycelium is used as cattle feed.

The basic citric acid solution is passed through the water to the chemical plant to separate the citric acid.

Separation of citric acid and obtaining it in crystalline form. Once the mycelium is separated, the culture fluid contains a mixture of citric, gluconic and oxalic acids (shavel (amber) acid), sugar precipitates and mineral mixtures.

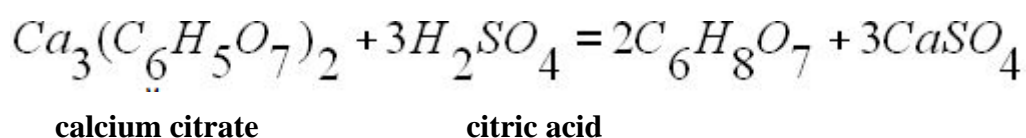
The separation of citric acid from the culture fluid is based on the fact that it forms a low solubility property in the three calcium salts of citric acid.

The neutralization process is carried out on a special device - a neutralizer, which in turn is equipped with a mixer and steam batteries. The culture liquid is heated to boiling point and lime or lime milk is added slowly under continuous stirring.

Neutralization is completed when the pH of the culture medium is 6.8-7.5. In this case, salts of all three acids are formed. [4]

Calcium citrate and oxalate precipitate, calcium gluconate and mineral salts remain in solution. Calcium citrate and oxalate are separated from the solution in a vacuum filter and thoroughly washed in hot water. Calcium citrate is mixed with a certain amount of water, poured into the reactor, and activated charcoal is added to it (as a sediment). The reactor is then heated to 60 ° C and a certain amount of sulfuric acid is poured into it during mixing.

The mixture is boiled for 10-20 minutes. Calcium citrate reacts with sulfuric acid according to the following equation, and citric acid is released in pure form:



Calcium oxalate is not degraded under these conditions. Once the calcium citrate is completely separated, granulated barium sulfate is added to the reactor to precipitate heavy metals. The citric acid solution is separated from the residues of gypsum, calcium oxalate, coal and heavy metal salts by a vacuum filter. The filtered citric acid solution is directed to evaporation. Evaporation in vacuum equipment is carried out in two stages.

In the first equipment, the solution is evaporated to a density of 1.24-1.26 g / cm³, and the gypsum residue precipitates. After separation of gypsum in the dense filter, the clear solution is evaporated in the second device to a density of 1.35–1.36 g / cm³. The amount of citric acid in the solution is 80%.

CLEAR CONCLUSIONS AND PRACTICAL SUGGESTIONS. The solution evaporated in a vacuum device at a temperature of 70 ° C is fed to the crystallizer. In the crystallizer, the solution is cooled to a temperature of 35-37 ° C, resulting in the formation of citric acid crystals. Crystallization is accomplished by continuous stirring and gradual cooling to 8–10 ° C. The resulting citric acid crystals are separated by centrifugation and washed in a small amount of cold water and sent for drying.

Drying of citric acid in the crystalline state is carried out in a pneumatic dryer with a tape or drum, at a temperature not exceeding 35 ° C.

The finished drug should contain not less than 99.5% of citric acid (in terms of monohydrate).

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